

Rebuttable Presumption against Registration
Decision Document on Maleic Hydrazide

(U.S.) Environmental Protection Agency
Washington, DC

Jun 82



BIBLIOGRAPHIC INFORMATION

PB82-240300

Rebuttable Presumption against Registration: Decision
Document on Maleic Hydrazide.

Jun 82

PERFORMER: Environmental Protection Agency, Washington, DC.
Office of Pesticides and Toxic Substances.
EPA-540/9-82-010

This document details the final determination regarding the Rebuttable Presumption Against Registration (RPAR) of products containing the above chemical. The responses by EPA to comments on the preliminary determination by the Scientific Advisory Panel and the U.S. Department of Agriculture are contained in this document. A bibliography of citations used in this final determination is also provided.

KEYWORDS: *Pesticides, *Environmental surveys, *Rebuttable
Presumption Against Registration, *Maleic
hydrazide.

Available from the National Technical Information Service,
Springfield, Va. 22161

PRICE CODE: PC A03/MF A01

REPORT DOCUMENTATION PAGE		1. REPORT NO. EPA 540 9-82-010	2.	3. PB82-240300
4. Title and Subtitle Pesticide Rebuttable Presumption Against Registration Decision Document on Maleic Hydrazide			5. Report Date March 1982	
7. Author(s) USEPA Special Pesticide Review Division			8. Performing Organization Rept. No.	
9. Performing Organization Name and Address U.S. Environmental Protection Agency (TS-791) 401 M Street SW Washington, DC 20460			10. Project/Task/Work Unit No.	
			11. Contract(C) or Grant(G) No. (C) (G)	
12. Sponsoring Organization Name and Address Same as above			13. Type of Report & Period Covered	
			14.	
15. Supplementary Notes This document details the final determination regarding the				
16. Abstract (Limit: 200 words) This document details the final determination regarding the Rebuttable Presumption Against Registration (RPAR) of products containing the above chemical. The responses by EPA to comments on the preliminary determination by the Scientific Advisory Panel and the U.S. Department of Agriculture are contained in this document. A bibliography of citations used in this final determination is also provided.				
17. Document Analysis a. Descriptors Decision document on Maleic Hydrazide				
b. Identifiers/Open-Ended Terms				
c. COSATI Field/Group				
REPRODUCED BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161				
18. Availability Statement		19. Security Class (This Report)		21. No. of Pages
		20. Security Class (This Page)		22. Price

REBUTTABLE PRESUMPTION AGAINST REGISTRATION

DECISION DOCUMENT

ON

MALEIC HYDRAZIDE

Office of Pesticides and Toxic Substances
Office of Pesticide Programs
United States Environmental Protection Agency
Washington, D.C.

June, 1982

TABLE OF CONTENTS

	Page
I. INTRODUCTION	
A. General Background	1
B. Regulatory Background	
1. Grassley-Allen Amendment to FIFRA	1
2. Data Call-In	2
3. Suspension of DEA-MH Registrations	3
C. Chemistry and Registration Information	
1. Use and Usage	3
2. Tolerances	3
3. Chemistry	4
II. EVALUATION	
A. Oncogenicity	
1. Original Supporting Data	5
2. Reassessment of Original Supporting Data	5
3. Negative Results Studies Reported in PD 1	7
4. Additional Negative Results Studies	8
5. Studies Reported Since Publication of PD 1	8
6. Hydrazine Contamination	9
7. Conclusion	10
B. Reproductive and Teratogenic Effects	
1. Original Supporting Data	12
2. Reassessment of Original Supporting Data	12
3. Studies Reported Since Publication of PD 1	13
4. Conclusion	13
C. Mutagenicity	
1. Original Supporting Data	14
2. Reassessment of Original Supporting Data	15
3. Studies Reported Since Publication of PD 1	16
4. Conclusion	20
III. DECISION	
A. Decisions and Supporting Rationale	28
B. Additional Data Requirements	29
IV. BIBLIOGRAPHY	

I. INTRODUCTION

A. GENERAL BACKGROUND

Maleic hydrazide (MH) is a systemic plant growth regulator registered and used in the United States for growth control of tobacco, potatoes, onions, non-bearing citrus, grasses, trees and shrubs. There are two end-use formulations, the diethanolamine (DEA-MH) salt and the potassium (K-MH) salt.

MH was referred to the Agency for Rebuttable Presumption Against Registration (RPAR) review in October, 1976. The referral was based on laboratory studies which indicated that MH induced oncogenic, mutagenic and reproductive effects. In October, 1977, the Agency initiated an RPAR review by publishing a Position Document 1 (PD 1) for MH. The PD 1 described studies which met or exceeded the RPAR criteria for oncogenic, mutagenic and reproductive effects.

Since the issuance of PD 1 the Agency has reevaluated the cited studies and additional data obtained since PD 1. Based on this review the Agency has determined that insufficient evidence exists to justify continuation of the RPAR review. Consequently, all registered products containing MH, K-MH or DEA-MH will be returned to the registration process.

This decision document reviews the currently available information concerning the potential health effects of MH and provides a rationale for the decision to return MH to the registration process. This document is organized into three sections:

The Introduction presents general background, regulatory background and chemistry and registration general information concerning maleic hydrazide and its salts.

The Evaluation presents a reevaluation of toxicological evidence which supported the initiation of the RPAR investigation in 1976. Each area of toxicological concern (oncogenicity, mutagenicity and reproductive effects) is analyzed separately by first presenting the evidence which supported the initiation of the RPAR followed by a reevaluation of that evidence along with analyses of any new information since 1977. The final portion of each section of the Evaluation is a conclusion stating the Agency's position on that area of toxicological concern.

The Decision section presents the rationale for and conditions under which the maleic hydrazide RPAR investigation is being terminated.

B. REGULATORY BACKGROUND

1. Grassley-Allen Amendment to FIFRA

After Position Document 1 (PD 1) was published in October 1977, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) was amended in 1978. One major feature of these 1978 Amendments was Section (3)(c)(8), otherwise known as the Grassley-Allen amendment. Briefly, this amendment prohibited initiation of formal action (such as an RPAR review) unless the action is based on a validated test or other significant evidence raising

prudent concerns of unreasonable adverse risk to man or the environment. A validated test was defined as one which is conducted and evaluated in a manner consistent with acceptable scientific procedure; "other significant evidence" was defined as that which relates to the uses of a pesticide and its adverse risk to man or the environment. It was intended that "other significant evidence" of adverse risk means factually significant information and was not to include evidence based on misuse of the pesticide unless the misuse is a commonly recognized practice. In determining whether a particular pesticide raises "prudent concerns," the Agency was directed to examine the degree of toxicity of the pesticide, as well as the likelihood of human and environmental exposure. Now the Agency applies this approach to all its risk triggers, including oncogenicity and mutagenicity triggers which do not on their face take exposure into account. This approach allows the Agency to preclude or end an RPAR proceeding in those situations in which the uses of the pesticide at current levels of exposure do not appear to pose an unreasonable risk to man or the environment.

Although Section (3)(c)(8) was not law before the maleic hydrazide PD 1 was published, it is the focal point and underlying foundation of this document. As will be shown in subsequent portions of this decision document, reanalyses of the original supporting data and consideration of data available after publication of Position Document 1 indicate that "validated tests and other significant evidence" may not have existed to support the initiation of an RPAR action on maleic hydrazide and its two formulations, DEA-MH and K-MH.

Generally, after PD 1 is published the Agency proceeds to write and publish a Position Document 2/3. This document is a consideration and analysis of all comments solicited by PD 1 in addition to a use by use risk/benefit analysis. Position Document 2/3 is designed to propose actions, if any, for the continued use of the chemical. Actions are based on and supported by the thorough analyses of comments, risks and benefits. Clearly, the absence of any of the analyses would make it difficult to proceed with an appropriate and just review of the chemical. It was this absence of clear risk analyses for oncogenic, mutagenic and reproductive effects that halted production of PD 2/3 for maleic hydrazide. Agency scientists could not adequately assess the risk potential of maleic hydrazide because of the paucity of valid toxicological information and conflicting results within each data base (oncogenicity, mutagenicity and reproductive effects).

2. Data Call-In Under FIFRA Section (3)(c)(2)(B)

To fill these toxicological gaps, the Agency required all maleic hydrazide registrants to supply data regarding the reproductive effects and mutagenic potential of DEA-MH and K-MH. The Notice and Order announcing these additional data requirements for maleic hydrazide was sent to all registrants on August 14, 1980. Oncogenic effects testing was not requested since there were studies already underway at the National Cancer Institute and other national and international research facilities.

Failure to comply with data requests under FIFRA Section (3)(c)(2)(B) can result in the suspension of registrations. On February 26, 1981, Uniroyal Chemical Incorporated agreed to initiate a two generation reproductive effects study on rats with K-MH, as well as a series of mutagenicity tests on this formulation. To date, however, no registrant or other interested party has agreed to provide similar data for (DEA-MH).

3. Suspension of DEA-MH Registrations

Because manufacturers of DEA-MH decided not to submit the required additional data, the Agency suspended all DEA-MH registrations. This suspension action became effective 30 days after manufacturers received the notice in October, 1981. Along with the suspension, the Administrator granted that manufacturers may dispose of their existing stocks. Existing stocks, for the purpose of the suspension action, were defined as those DEA-MH products which were packaged, labeled and released for shipment on or before the effective date of the suspension. The suspension action in no way prohibits the use of DEA-MH products after it took effect in November, 1981, nor does it prohibit or restrict the manufacture of DEA-MH products for export purposes only. The suspension action was not a cancellation and may be lifted at any time if there is compliance with the August 14, 1980 data request.

C. CHEMISTRY AND REGISTRATION INFORMATION

1. Use and Usage

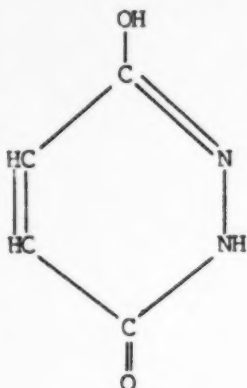
Maleic hydrazide is a systemic plant growth regulator which is translocated within the plant and controls unwanted growth by inhibiting cell division. Two formulations, the diethanolamine salt (DEA-MH) and the potassium salt (K-MH) are currently registered for growth control needs on tobacco, potatoes, onions, non-bearing citrus trees, grasses, trees and shrubs. Of the 1,841,950 gallons of maleic hydrazide products sold in 1980, approximately 88 percent was used on tobacco, 9 percent was used on potatoes, 1 percent was used on onions with the remainder being used on grasses, trees and shrubs. The 1980 sales records show that the potassium formulation was the preferred product for all uses (Thies, 1981a).

2. Tolerances

Pesticide tolerances for residues of MH in or on raw agricultural commodities have been established at 50 ppm in or on potatoes and 15 ppm in or on onions (dry bulb) (40 CFR 180.175). A food additive tolerance of 160 ppm is established for residues of MH in the processed commodity, potato chips (21 CFR 121.1006). Since the use of MH on tobacco plants is classified as a "non-food" use, no tolerance is necessary.

3. Chemistry

Maleic hydrazide (1,2 Dihydropyridazine-3,6-dione or 6-hydroxy-3(2H)-pyridazinone) has an empirical formula of $C_4H_4N_2O_2$; molecular weight, 112.09; elemental analysis, C-42.86%, H-3.60%, N-24.99%, O-28.55% and molecular structure as follows (Farm Chemicals Handbook, 1981):



Maleic hydrazide is a white crystalline powder with a melting point of 292°C , and decomposes at over 300°C . It is stable to hydrolysis but is decomposed by strong oxidizing agents with release of nitrogen. Maleic hydrazide behaves as a mono-basic acid and forms salts; its alkali metal (potassium) and amine (diethanolamine) salts are water soluble (0.6% at 25°C) and slightly soluble in hot alcohol (0.1% at 25°C).

Maleic hydrazide products are marketed under several trade names; some of which are MH-30®, Slo-Gro®, Royal Slo-Gro®, Slows-It®, De-Sprout®, De-Cut®, Fair Plus®, Sprout Stop®, and Super Sucker-Stuff®.

II. EVALUATION

In this portion of the document the studies which supported the original RPAR action will be presented first. Following this will be the Agency's reassessment of original data, as well as analyses of new data since publication of PD 1, and an overall conclusion. Agency reassessments are based upon either new evidence presented since the publication of Position Document 1, updated criteria for judging the validity of studies, or both.

A. ONCOGENICITY

1. ORIGINAL SUPPORTING DATA

Position Document 1 cited three rodent studies supporting the positive oncogenic potential of maleic hydrazide: Epstein and Mantel (1968), Barnes et al. (1957), and Dickens and Jones (1965). The Agency's Carcinogen Assessment Group (CAG) concluded from these studies that maleic hydrazide was oncogenic in mice, and therefore warranted the initiation of an RPAR action (CAG, 1977).

2. RE-ASSESSMENT OF ORIGINAL SUPPORTING DATA

a. Epstein and Mantel (1968)

In the study by Epstein and Mantel (1968), infant male and female Swiss ICR/Ha mice received a subcutaneous injection of maleic hydrazide the free acid. Doses of 5, 10, 20 and 20 mg maleic hydrazide as a suspension in tricapylin were given on day 1, 7, 14 and 21, respectively, to 193 mice. Another group of 67 mice were similarly treated with doses of 0.5, 0.5, 1.0 and 1.0 mg maleic hydrazide in aqueous solution. A group of 170 mice served as solvent controls. Total doses of maleic hydrazide given to the infant mice were 7,883 mg/kg in suspension and 517 mg/kg in aqueous solution. Hence, the infant mice were given tremendous amounts of maleic hydrazide, particularly in suspension, with each treatment. Mortality was quite high among test animals and appears to reflect the high doses used. Surviving mice were necropsied at 49 to 51 weeks and tissues and lesions were examined histologically. Subsequent to publication of PD 1, the Agency received confirmation from Uniroyal that the maleic hydrazide tested by Epstein and Mantel contained 4000 ppm hydrazine.

An increased incidence (number affected/number survivors) of hepatomas in treated male mice surviving at least 49 weeks was found and is as follows: 4/48 (8%) in controls, 3/17 (18%) in males given maleic hydrazide aqueous solution and 19/26 (65%) in males maleic hydrazide in tricapylin suspension. Prior to 49 weeks, hepatomas were also found in two other male mice given suspension. Hepatomas were not observed in female mice. Local fibrosarcomas were found in one treated female and one treated male.

Hydrazine sulfate has been shown to produce hepatomas in CBA/Co/Se mice, when administered by gavage at a total dose as low as 42 mg (Biancifiori, 1970 ; see section on Hydrazine Contamination). These results provide evidence that hydrazine could have contributed to hepatoma formation in the infant mice treated with maleic hydrazide as reported by Epstein and Mantel. However, a quantitative estimate of the effect of hydrazine as an impurity in the study by Epstein and Mantel is limited by differences in experimental design of the two studies. Pure hydrazine has not been tested by the method of Epstein and Mantel, namely by an unusual method of injecting high doses of maleic hydrazide into infant mice. This makes interpretation of their results unclear in relation to lower lifetime exposures by other routes.

b. Barnes et al. (1957)

Barnes et al. (1957) assessed the carcinogenicity of maleic hydrazide (MRC 41 as a dry powder) fed to rats and mice at a level of 9% in the diet for 100 weeks. They also evaluated the carcinogenicity of maleic hydrazide (monosodium salt) given to mice and rats by weekly subcutaneous injections of 500 mg/kg in aqueous solution for 100 weeks. Twelve to 15 animals of each sex were assigned to each treatment group, and 10 animals of each sex comprised each control group. Average survival times in control and treated groups were 61 to 88 weeks in rats and 74 to 94 weeks in mice. Six or fewer animals per group survived 100 weeks. Nine to 15 rats and 3 to 9 mice in each group were examined histopathologically.

Maleic hydrazide did not affect survival or growth of the animals in this study. Tumor incidence was not significantly increased in treatment groups with the exception of total tumor incidence in female mice in the feeding study. No tumors were diagnosed in female control mice (four examined histologically, three examined by necropsy only) compared to one malignant tumor, one lymphoma, and five benign tumors in female mice fed 10,000 ppm maleic hydrazide (eight examined histologically, six examined by necropsy only). Tumor incidence was reported as the combined total incidence for all treatment groups. Tumors found in treated mice included three lymphomas, one lung carcinoma, one hepatoma, one sarcoma and 10 lung adenomas. However, it is unclear in the study report which tumors were specifically found in female mice given maleic hydrazide in the diet. In comparison, combined tumor incidence in all combined control groups of mice included four lymphomas, five lung adenomas and one hepatoma. In addition, it was not reported whether the maleic hydrazide test samples contained hydrazine. Maleic hydrazide was not shown to be a

carcinogen in any particular target organ in the study by Barnes et al. (1957). Thus, the results of the study by Barnes et al. (1957) do not appear adequate to provide a conclusion on the carcinogenicity of maleic hydrazide. These authors (Barnes et al., 1957) also reported negative results in mice simultaneously treated with maleic hydrazide and croton oil on the skin and in mice given pellet implants in the bladder of 20% maleic hydrazide in cholesterol.

c. Dickens and Jones (1965)

In the study by Dickens and Jones (1965), six male rats (100 g; closed outbred Wistar Colony rats) were given subcutaneous injections of 2 mg maleic hydrazide (free acid) suspension in arachis oil in the flank twice weekly for 65 weeks. Treated rats were compared with six male vehicle controls. Rats were allowed to survive for 106 weeks and each rat was necropsied.

Three treated rats had local sarcomas (one sarcoma and two fibrosarcomas) and one of the three rats had a hepatoma. The local sarcomas were detected at 84, 95 and 104 weeks and found to be transplantable. Local tumors were not found in vehicle controls. The comparison of local sarcoma incidence between groups was not significant ($P = 0.091$). A group of rats treated with maleic hydrazide in aqueous solution was not evaluated in this study to allow an estimate of the possible influence of the physical nature of the oily suspension on local tumor formation. For example, local tumors were not induced in the injection experiment by Barnes et al. (1957). The ability of the Dickens and Jones (1965) study to support a conclusion of carcinogenicity for maleic hydrazide is further weakened by the absence of a clear demonstration of the effects of maleic hydrazide on a target organ other than at the site of injection. In addition, there is the failure of Hunter et al. (1975) to repeat their results in a similar study with a larger number of animals.

3. NEGATIVE RESULTS STUDIES REPORTED IN POSITION DOCUMENT 1

Hunter et al. (1973) repeated the study of Dickens and Jones (1965) with the same protocol and using the same strain of rat. Treatment and vehicle control groups consisted of 25 male and 25 female Wistar rats. Each animal was subjected to necropsy, and injection sites and all lesions were examined microscopically. Median survival was greater than 85 weeks although chronic respiratory disease incidence was high. Tumor incidences were not significantly increased by treatment with maleic hydrazide or DEA-MH.

Innes and co-workers (1969) tested two strains of male and female mice for tumorigenic effects of maleic hydrazide (not reported as free acid or salt). Mice were fed 1,000 mg/kg/day by gavage between 1 and 4 weeks of age. In addition, a maintenance

dose of 3,000 ppm maleic hydrazide was fed in the diet until the end of the 18 month study. No significant increase in tumor incidence was reported in any of the test groups.

Mukhorina (1962) tested maleic hydrazide for its tumor inducing potential by giving both male and female mice 1 mg maleic hydrazide (in saline) once weekly by subcutaneous injections or 0.5 mg maleic hydrazide (in saline) twice weekly by gavage for 27 months. No increased incidence of tumors was reported for any of the test groups. No histopathology was reported.

4. ADDITIONAL NEGATIVE STUDIES

Mannel and Grice (1975) fed maleic hydrazide, at a 2% level in the diet, to rats in a study which only lasted 26 weeks. No carcinogenic effect was reported. In another study (Food Research Laboratories, 1955), male and female rats were given dietary levels of 0.5, 1.0, 2.0 or 5.0% maleic hydrazide (sodium salt) or 0.1% maleic hydrazide (diethanolamine salt) for twelve weeks. No increased tumor incidence above the control values was reported for either experimental group; however, survival was poor and only tissues from one to six animals per group were examined microscopically.

5. STUDIES REPORTED SINCE PUBLICATION OF PD 1

a. International Research and Development Corporation (1979)

In March, 1978, the International Research and Development Corporation began a 24-month carcinogenicity study sponsored by Uniroyal Incorporated. The study was completed recently and the final report is currently in review.

In this study, male and female CD-1 mice were given maleic hydrazide (potassium salt) at levels of 1,000, 3,000 or 10,000 ppm in the diet. A 12-month interim report dated December 11, 1979 has been made available to the Agency for review. The study appears to have been adequately conducted. Interim results show that the survival rate is high and that proper levels of maleic hydrazide were being fed to the animals. Also, the interim report states that no compound-related effects on hematologic test results, body weights, survival, food consumption, appearance or behavior were noted. A conclusion as to the carcinogenicity of maleic hydrazide (potassium salt) under the conditions of this study must await review of the final report, including a complete description of experimental details and results, and pathology on all animals.

b. Van Der Heijden et al. (1981)

Van Der Heijden et al. (1981) studied the effects of purified maleic hydrazide on Wistar rats. Male and female rats

were fed 0, 1 and 2 percent maleic hydrazide as a dietary supplement. The control (0%) and 1% experimental group contained 55 animals of each sex whereas the 2% experimental dose group contained 65 animals of each sex. Maleic hydrazide was purified and contained less than 1 ppm hydrazine, a known carcinogen (Biancifiiori, 1970).

Results show a significant reduction in the body weight of experimental female animals and also a slight but insignificant increase in mortality for females in both experimental groups. Gross and microscopic pathology of rats killed at 12 and 28 months did not reveal any non-neoplastic changes that could be related to treatment. The percentage of tumor bearing rats in control and treatment groups did not differ significantly.

c. Cabral et al. (1981)

In a study performed in collaboration with the Van Der Heijden study described above, Cabral et al. (1981) gave maleic hydrazide (99% pure, <1 ppm hydrazine contamination) to mice (C57, black strain) either subcutaneously (in tricapylin) on days 1, 7, 14 and 21 after birth at a total dose of 55 mg., or orally (in olive oil) at a weekly dose of 510 mg/kg for life. No significant increase in tumor incidence was observed under these experimental conditions (WHO, 1980 Annual Report, Abstract; Thies, 1981b). However, no final report has been available for review by the Agency. Except for the strain of mice used, the Cabral experiment using purified maleic hydrazide (hydrazine less than 1 ppm) was identical to the Epstein et al. study (1968) which used maleic hydrazide with a hydrazine contaminant level of approximately 4,000 ppm to treat random-bred Swiss mice (ICR/Ha).

6. HYDRAZINE CONTAMINATION

Hydrazine contamination may be a key factor in making a reasonable determination of the oncogenic potential of maleic hydrazide. As previously pointed out, two similar studies (Epstein and Mantel, 1968; Cabral et al. 1981) show different results. A primary difference between the two studies is the level of hydrazine sulfate contamination: 4,000 ppm in the Epstein study and less than 1 ppm in the study by Cabral.

Several studies show a definite relationship between hydrazine sulfate and tumor induction. One study, Biancifiiori (1970), shows a dose-related induction of hepatomas in male mice treated by gavage with hydrazine sulfate in aqueous solutions. Mice were dosed once daily, 6 days per week for 25 weeks. After 100 weeks, each animal was necropsied and tissues were further examined microscopically. Hepatoma incidences are shown in the Table.

Table I

Daily Dose of Hydrazine Sulfate* (mg/kg/day)	Number of Animals with Hepatomas
0	3/30
1	1/25
2	7/25
4	12/25
8	15/25

*Doses were administered as 0.14, 0.28, 0.56 and 1.15 mg/day hydrazine sulfate. Assuming a mouse body weight of 35 g, these doses are equivalent to 4, 8, 16 and 32 mg/kg/day of hydrazine sulfate. Comparison of molecular weights for hydrazine sulfate (130.13) and hydrazine (32.05) shows that hydrazine itself was 25% of each hydrazine sulfate dose.

Oncogenic risk to mice from exposure to hydrazine in this study can be calculated using the linearized multistage model. Using the above data, the lifetime risk to mice due to hydrazine alone corresponding to a 1 ppm contaminant level of hydrazine is 8.88×10^{-6} (Anderson, 1981). In comparison, the oncogenic risk to mice from hydrazine at the equivalent contamination level in the Epstein study (4,000 ppm hydrazine) is 3.6×10^{-2} . Clearly, when comparing the Epstein and Cabral studies, or any other oncogenicity studies, the level of hydrazine contamination is crucial.

Since hydrazine is oncogenic and is a contaminant of maleic hydrazide products, the Agency considered the lifetime oncogenic risks to humans using the one-hit model and the Biancifiori (1970) male mouse liver data. For dietary exposure, residues were considered to be at tolerance levels with 100% of registered crops treated. For applicator exposure, worst case residues were calculated assuming 100% dermal absorption. In each case, and assuming that hydrazine contamination is at 15 ppm,** the highest level reported in registered MH products, the estimated oncogenic risks were very low.

** 15 ppm is the highest level of hydrazine reported to be contained in currently registered technical MH products.

7. CONCLUSION

Based on reassessment of the three original supporting studies (CAG, 1981), the Agency concludes that previously available evidence is inadequate to support continuation of the oncogenicity trigger. This conclusion takes into account the following considerations in the interpretation of the original supporting data.

Results of the key study cited in PD 1 by Epstein et al. (1968) are considered inconclusive because of the possible effect of the carcinogenic hydrazine contaminant (at 4000 ppm) on the carcinogenic response. Information about the hydrazine contamination level was not available to the Agency when PD 1 was published. Furthermore, the unusual testing regimen, involving dosing of infant mice with extremely high doses of test chemical, by injection prevents use of this study for quantitative risk assessment.

Studies cited in PD 1 as supportive of the original presumption are also flawed. Comparison of total number of tumors in control and treatment groups (Barnes et al., 1957) is an inadequate basis from which to draw conclusions about carcinogenicity; a carcinogenic effect on specific target organs was not observed by Barnes et al. (1957). Results of the study by Dickens and Jones (1965) in which tumor formation was localized at injected sites, furnish inconclusive evidence for maleic hydrazide carcinogenicity because tumors in distant target organs were not observed and the results were not repeated in a similar study by Hunter et al. (1973).

The importance of the Epstein study used in the original presumption of oncogenicity of maleic hydrazide has been reevaluated by the Agency in light of the high contamination level of hydrazine (4,000 ppm). In this vein, the Agency has determined that current formulations of maleic hydrazide used in registered pesticide products have levels of hydrazine which are toxicologically insignificant with respect to oncogenic risk (See discussion of hydrazine contamination). These considerations and the weaknesses of the supportive studies cited above have provided the reasons for the Agency's determination to withdraw maleic hydrazide from further RPAR review pending evaluation of forthcoming oncogenicity studies in context with other studies requested from the registrant.

B. REPRODUCTIVE AND TERATOGENIC EFFECTS

1. ORIGINAL SUPPORTING DATA

Position Document 1 cited several studies which discussed the reproductive effects of maleic hydrazide on mammalian and non-mammalian systems. Only one study (Oser, 1955) supported the initiation of the RPAR review based on adverse reproductive effects of maleic hydrazide. In Position Document 1, the Agency concluded that 1) DEA-MH produces significant adverse effects on several reproductive parameters and 2) an ample margin of safety could not be demonstrated as to the reproductive effects produced by DEA-MH. For these reasons, an RPAR review was initiated for DEA-MH, but not for K-MH or the parent compound maleic hydrazide.

Food Research Laboratories (Oser, 1955) performed a three generation reproductive study on albino rats (strain not reported) by administering 0.5, 1.0, 2.0 and 5.0% sodium maleic hydrazide (Na-MH) and 0.1% and 1% diethanolamine maleic hydrazide in the feed. The study presented data on the number of matings, number of litters born alive, average number of pups per litter, average weight of the pups at weaning, and various other indices such as fertility, gestation, viability and lactation. Results showed that while the Na-MH dosed rats displayed no adverse reproductive effects at any dose (0.5, 1.0, 2.0, 5.0%), the 0.1% DEA-MH dosed rats exhibited significantly decreased fertility, viability and lactation in the first generation. The 1% DEA-MH dosing was discontinued after 12 weeks due to high mortality.

Based on the results of the Oser study, the Agency expressed concern about the proximity of the estimated exposure level of maleic hydrazide (5.5 mg/day, based on tolerance) to the observed reproductive effect level in man (6,000 mg/day; extrapolated from Oser results). The Agency was also concerned that the Oser study did not have adequate data to establish a dose response relationship or a no-observed-effect-level for DEA-MH.

2. RE-ASSESSMENT OF ORIGINAL SUPPORTING DATA

The study (Oser, 1955) upon which the reproductive effects concerns, expressed in Position Document 1 were based, has several deficiencies.

The data in the Oser study were reported to the Agency in summarized form and the original individual raw data points were not available for Agency review. In addition, no statistical analysis of these data was reported, and although the number of animals per group was apparently twelve, this number is not clearly given in either the text or the tables.

The Oser study reported no adverse reproductive effects for Na-MH dosed animals, although weaning weights of the pups (sex of offspring not indicated) ingesting 5% Na-MH were lower in all three generations than those of the controls. The investigator suggested that the reduction in weaning weights of the pups resulted from a problem with the palatability of high levels of Na-MH since this reduction was not observed at the lower dosage levels. However, no independent confirmation can be made since data were not reported completely and no statistical analysis was provided.

Oser did report adverse reproductive effects for DEA-MH dosed animals. The author stated that a reduction in the average number of pups per litter occurred with a reduction in indices of fertility, viability and lactation. Statistical analysis of the summarized data have been performed by Agency scientists (Hill, 1977; Chen, 1981). At the 0.1% DEA-MH dose level the indices of fertility, viability and lactation are significantly different from that of control (p less than 0.05) when using Chi square analysis and assuming that the denominator of the various indices is constant (Hill, 1977; Chen, 1981). Also, the proportion of animals surviving to weaning is statistically reduced if analyzed in this fashion (Chen, 1981). The adverse effects of the DEA-MH are also manifested in toxicity of adult animals with growth depression and high rates of mortality. The author reported that these signs of toxicity in adult animals were probably due to the DEA moiety because DEA administered alone at the 1% level produced similar responses in growth and mortality as 1% DEA-MH. However, this observation was reported only in the written text of the report and no data were presented to support this conclusion. In addition, the effect of the DEA moiety alone on reproductive capacity was not examined and the study design is questionable since survivors of acute toxicity tests were permitted to continue on the same dietary regimes for the purpose of conducting a reproduction assay (Chitlik, 1981).

3. STUDIES REPORTED SINCE THE PUBLICATION OF PD 1

Only one study has been located in the scientific literature, since publication of Position Document 1, which evaluates either the teratogenic or adverse reproductive potential of maleic hydrazide. Khera et al. (1979) evaluated the teratogenic effects of 400, 800, 1200 and 1600 mg/kg/day doses of maleic hydrazide (free acid) given by intubation (on days 6-15 of gestation) to Wistar rats (20 mated females per experimental group).

Of the many customary observations used for evaluation (i.e., number of implants per pregnancy, number of females with resorption, total number of resorptions, number of dead fetuses, fetal weight, number of anomalous fetuses per number examined, number of litters with abnormalities per number examined), no endpoint at any dosage level differed significantly from that observed in the control group. The authors analyzed the data statistically using test comparisons assigning the significant level at p less than 0.05. No signs of toxicity were reported at any of the dose levels used. The raw data from this study have been reviewed by Agency scientists and the lack of statistical significance between control and experimental groups has been confirmed (Chen, 1981).

4. CONCLUSION

Based on the reassessment of the single supporting study (Oser, 1955) the Agency concludes that there is inadequate evidence to support continuation of the reproductive effects trigger for DEA-MH. This conclusion is based on inadequacies in design and execution of the Oser work. It should be noted, however, that while the reproductive effects trigger for maleic hydrazide cannot be supported, the available information, including the negative teratogenic effects reported by Khera (1979), is still inadequate to make a final assessment that this chemical does not induce adverse reproductive effects and or possess teratogenic potential. Therefore, it is necessary to review the forthcoming study (Raltech, 1981) before the adverse reproductive

and development potential of maleic hydrazide can be adequately assessed. In May, 1981, Raltech Scientific Services began a two generation reproductive effects study of K-MH using Charles River CD® SD(BR) rats. This study is being sponsored by Uniroyal Incorporated in fulfillment of their responsibilities set forth in the Agency's data request under FIFRA Section (3)(c)(2)(B). The study should be available for Agency review in late 1982.

C. MUTAGENICITY

1. ORIGINAL SUPPORTING DATA

Position Document 1 cited several studies, summarized below that supported the initiation of an RPAR review based on the mutagenic effects of maleic hydrazide. These studies, considered as multitest evidence, indicated that maleic hydrazide produces chromosome aberrations in plants and animals (plants being more sensitive) and may induce point (gene) mutations in procaryotic systems, plants and insects.

a. Manna and Parida (1972)

These researchers examined meiotic chromosomes from several grasshopper species which were given a dose of 5 mg maleic hydrazide per individual in food. The mortality rate 48 hours after feeding ranged from 17 to 58%, depending on the species; none of the controls died. In treated males chromatid and chromosome breaks were noted at various stages of meiosis; chromosome bridges and lagging chromosomes were seen in anaphase I and II figures; and chromosome stickiness was prevalent in all meiotic stages examined. In control males, no breaks were found, and chromosome stickiness was much less than in treated animals.

b. Manna and Das (1976)

Manna and Das (1976) injected mice with maleic hydrazide (167 mg/kg body weight) and then examined their bone marrow for chromosome aberrations 10 to 120 hours after treatment. The frequency of gaps, chromatid breaks and translocations was significantly increased in treated animals. An increase in isochromatid breaks was not noted, a fact which may indicate that maleic hydrazide does not affect cells in the G₁ phase of the cell cycle.

c. Nasrat (1967)

Nasrat (1967) reported that maleic hydrazide induced a significant increase ($p < .003$) in the incidence of sex-linked recessive lethals in Drosophila melanogaster. Although Position Document 1 pointed out two possible drawbacks to this study (the number of X chromosomes tested was only 1024, and the raw data were not available to rule out clustering of mutants within a few originally treated males), the results were considered adequate to partially support an RPAR action.

d. Northrop (1963)

Maleic hydrazide was used to treat a lysogenic strain, B. megatherium, in order to test the mutagenic capabilities of this chemical. Results show that maleic hydrazide was effective in increasing the proportion of virus-producing cells and streptomycin-resistant cells in B. megatherium cultures. This researcher surmised that the increase in mutation rate was caused, not by any specific reaction between the mutagen and the nucleic acid, but by some non-specific reaction such as denaturation.

e. Kak and Kaul (1975)

This study was undertaken to investigate the mutagenic activity of hydrazine hydrate in barley seeds. Treatments were administered alone or in the presence of maleic hydrazide. Results show that maleic hydrazide reduced seed germination and induced seed sterility to varying degrees.

2. REASSESSMENT OF SUPPORTING DATA

Studies cited in Position Document 1 clearly indicated that maleic hydrazide is genetically active in higher plants as well as in selected bacterial strains and some insects. The genetic activity of the compound in plants, however, is not unusual since maleic hydrazide functions by inhibition of cell division, thus retarding growth (Farm Chemicals Handbook, 1981). Thus, while many studies clearly indicate positive mutagenic effects in plants, they can also be considered as studies which support the efficacy of the compound. Although the Position Document 1 support of genetic activity in bacteria is adequate, the concern of similar activity in mammalian systems is not well founded and is certainly left open to serious scientific question.

Two of the original supporting studies (Manna and Das, 1976; Nasrat, 1967) have been declared invalid by the Agency (Mauer, 1981). Nasrat reported positive mutagenic activity in insects (D. melanogaster) while Manna and Das reported positive findings in mice.

The positive result reported by Nasrat (1967) (sex-linked recessive lethals in D. melanogaster) is flawed by lack of any compositional data (purity, source, etc.) on test substances. Most important, however, was the lack of any concurrent controls and the singular dose employed (0.4% MH mixed into the food). Furthermore, the reported spontaneous sex-linked recessive lethal frequency appears to be on the low side for the Oregon-K strain and thus serves to only enhance the reported positive results.

Manna and Das (1976) found chromosome aberrations in mice injected with maleic hydrazide. The Agency has invalidated this study because of the investigator's inadequate reporting (e.g., unstated number of animals per treatment group; inappropriate route of administration; no defined dosages; etc.). Also the strain of mice was not reported, therefore the background rate of aberrations is unknown. In addition, neither age nor sex of the animals was stated, the dosage of maleic hydrazide administered was unclear in the report, the site of the bone marrow selection for cells was not stated and there were no positive controls.

Position Document 1 also cited one study (McCarthy and Epstein, 1968) as reporting chromosome breaks occurring in cultured mouse fibroblasts. Although this study does report positive findings for cytotoxicity, mitotic inhibition and unbalanced growth due to maleic hydrazide, it fails to show that actual chromosome breaks occurred (Felkner, 1979).

Other studies reported in Position Document 1 show negative results for the genetic activity of maleic hydrazide in both mammalian and non-mammalian systems.

- a) Hitachi (1975) reported no increase in chromosome aberrations in liver cells of rats fed 1 percent maleic hydrazide in the diet.
- b) Barnes (1957) showed that sodium maleic hydrazide (NA-MH) did not inhibit mitosis in rats having Walker tumors.
- c) In a dominant lethal test in mice using DEA-MH, Epstein (1972) reported negative results (no evidence of increased chromosomal aberrations).
- d) Perry and Evans (1975) showed that treated fibroblasts derived from Chinese hamster ovaries revealed no compound related induction of chromosome aberrations or sister chromatid exchanges. The report further stated that in previous maleic hydrazide studies by these researchers no chromosome damage was ever noted in mammalian cells.
- e) McCann et al. (1975) and Andersen et al. (1972) both reported negative results for the *Salmonella typhimurium* histidine reversion test using maleic hydrazide. McCann used microsomal activation whereas Anderson did not.
- f) Szybalski (1958) reported that maleic hydrazide was not mutagenic in E. coli.

3. MUTAGENICITY STUDIES REPORTED SINCE THE PUBLICATION OF POSITION DOCUMENT 1 (See Table 2 at the end of this chapter.)

a. Chromosome Aberrations

An investigation performed by Litton Bionetics (1979) studied the effects of potassium maleic hydrazide (K-MH) in 7 to 8 week old Charles River CD-1 mice. K-MH (26% aqueous in KOH, pH 9) was administered orally according to both acute (0.5, 1.0 and 5.0 gm/kg) and subchronic (same dosages daily for 5 days) schedules. Triethylenemelamine (TEM) served as the positive control, and was administered as a single intraperitoneal injection of 0.3 mg/kg. It is important to note that, in contrast to the Manna and Das study (1976) in the same species, Bionetics reported no chromosome-breaking activity at doses up to 5000 mg/kg/day. Bionetics, however, did report significant transitory increases in hyperdiploidy at several treatment levels thus suggesting cell division abnormalities. The Bionetics study has been accepted as valid by Agency scientists (Mauer, 1981).

In other assays for chromosome aberrations, contradictory results were reported in the same test systems. Nawar and Hamza (1978) extended their studies in *Drosophila* to include testing for dominant lethals with negative results using MH alone; the authors also reported reductions in ethylmethanesulfonate-mutagenesis with combination treatment. On the other hand, Parkash and Miglani (1978) reported an increase in induced inversions as observed in third-instar larval salivary-gland chromosome preparations, following continuous feeding from egg eclosion at a level of 0.5% (5,000 ppm). No other types of chromosome aberration were reported.

The positive results reported by Parkash and Miglani are considered questionable, due to the lack of reporting test chemical purity, source and composition (especially regarding hydrazine contamination, if present), coupled with the lack of any details on the "control experiments" mentioned, but not otherwise explained. The negative results reported by Nawar and Hamza (1978) are also inconclusive because of the inadequate number of chromosomes tested (less than 1000 per brood). Further, although the source of maleic hydrazide is given, neither purity nor other physicochemical characteristics of the chemical's composition are reported.

In the only adequately reported mammalian cell (*in vitro*) assay for chromosome aberrations available for review, Nishi and colleagues (1979) found dose-dependent, increased chromosome breakage and other damage in Chinese hamster lung-derived V79 cells, following treatment with three MH preparations: the technical chemical at concentrations of 500 to 2,000 ug/ml K-MH; at 10,000 to 40,000 ug/ml; and, DEA-MH, at 5,000 to 20,000 ug/ml. The rate of breakage reached statistically significant maximal frequencies 3 to 5 times the control rate (4% of cells with chromosome aberrations), but only at cellular LD-50 values for MH and K-MH, and twice the LD-50 in the case of DEA-MH. Hydrazine and NaCl apparently had no effect on chromosome integrity at comparably high concentration (in the respective LD-50 ranges). The study does not provide conclusive evidence of chromosome aberrations, however, because of the highly toxic concentrations of maleic hydrazide required to achieve an induced clastogenic effect, and because no metabolic activation system was employed (V79 cells have rather poor capacity to metabolize xenobiotic chemicals). Further, no details on purity and other compositional features of the preparations were provided.

Both of the whole animal mammalian cytogenetic assays evaluated reported negative results for micronuclei in bone-marrow polychromatic erythrocytes of male Swiss mice injected intraperitoneally with maleic hydrazide preparations, even at organismal LD-50 levels (Chaubey et al., 1977; Paik and Lee, 1977). Whereas Chaubey and associates administered MH-free acid at maximal doses of 200 mg/kg, Paik and Lee employed DEA-MH at doses up to 600 mg/kg. While the Paik and Lee study is considered valid by the Agency, the Chaubey study is of questionable validity because of the insufficient number of animals used in all groups (4 per group).

b. Plant Versus Animal Metabolism

It has been suggested (Plewa, 1978) that mutagenically active substances may be generated in plants treated with certain pesticides. This could lead to significant genetic lesions as reported in plant tissues and possibly pose a potential for such hazard to exposed animals and humans. Although mutagenicity assays combining plant metabolism with

submammalian and mammalian test systems have been performed with a number of pesticides (e.g., the s-triazene herbicides, Plewa, 1978; ethylene dibromide, Scott et al., 1979), only one "adequate" study with maleic hydrazide has been identified (Benigni et al., 1979). Benigni and colleagues assessed forward mutation and somatic segregation in the fungus Aspergillus nidulans, exposed to homogenates prepared from cultured Nicotiana glauca cells which had been treated up to 12 days with maleic hydrazide (among five compounds tested in this system). The authors reported negative results for both types of genetic effect at a treatment level of 2 mg maleic hydrazide (per 12 ml N. glauca culture tubes), contrasted with singular positive results for both gene mutation (to 8-azaguanine resistance) and altered segregation (somatic crossovers, and nondisjunction) obtained with atrazine. It should be noted that all the chemicals tested, except MH, were readily metabolized by N. glauca cells.

The Benigni study is rendered invalid (and thus the reported negative result, inconclusive) by the many deficiencies in both experimental procedures and reporting. For example, neither the source of the chemical (or its purity, and other details on chemical composition), nor whether controls were run for the MH assay, was stated; also, the results of only a single dose level of MH are reported.

c. Hydrazine Contamination

A related issue for which some of these studies also generated information is the question of whether the presence of hydrazines as contaminants of maleic hydrazide preparations contribute to, or perhaps are wholly responsible for, the chemical's mutagenicity (Shiau, 1980; Tosk et al., 1979). For example, based upon a series of studies in an extremely sensitive strain of Bacillus subtilis, TKJ6321, it has been suggested that technical maleic hydrazide is mutagenic because of a contaminating hydrazine, the investigators suggesting the di-substituted 1,2-dimethylhydrazine form (Felkner, 1979). The mutagenic activity of technical maleic hydrazide, however, was found only when the test was performed without mammalian metabolic activation (with rat liver microsomes), and was not found in the presence of such activation (Shiau, 1980).

Although the presence of hydrazines, and/or metabolic conversion of maleic hydrazide preparations to hydrazine derivatives, may explain much of the mutagenic activity of these substances in plant tissue (Hartley-Asp, 1976; Ma, 1981; Schubert et al., 1979; Sturelid and Kihlman, 1975) and perhaps in certain animal studies reviewed (Nasrat, 1967; Nishi et al., 1979; Parkash and Miglani, 1978), demonstration of significant levels by analytical procedures was only rarely performed. One recent mutagenicity study in which such analytical procedures were used was that of Tosk et al. (1979), who tested a number of hydrazine derivatives, as well as pesticidal formulations of maleic hydrazide on Salmonella typhimurium strain TA 1530, which was known from previous work to be hydrazine sensitive. Tosk used the TA 1530 strain in lieu of the commonly used Ames strains TA 1535 and TA 100, which have proved insensitive to hydrazine mutagenesis. These authors reported that, although two formulations of maleic hydrazide, DEA-MH and K-MH, were mutagenic in TA 1530, technical maleic hydrazide was not. In the expectation that some interactions in the maleic hydrazide formulations might be responsible for their activity (compared

to MH technical), chemical analyses of all the maleic hydrazide preparations by the authors revealed concentrations of free hydrazines apparently too low (less than 10 ppm) to account for their mutagenicities. The authors were left with suggesting that perhaps TA 1530 cells themselves perform an activating biotransformation upon other ingredients in the formulations to produce mutagenic by-products.

Whereas the presence of DEA, a chemical which enhances the penetrating qualities of maleic hydrazide, may explain the mutagenicity of DEA-MH in TA 1530 cells, as reported by Tosk et al., that due to treatment with K-MH is puzzling. Although unstated by Tosk and his colleagues in their paper, it is conceivable that the K-MH employed in their study was the commercially available preparation, "Royal MH-30", which contains a surfactant as one of the inert ingredients. The surfactant may enhance the absorption of maleic hydrazide by the cells thus accounting for the increased mutagenic activity seen with K-MH treatment.

The study by Tosk and associates, however, is unacceptable as an adequate assay of MH mutagenicity. Although promised in future studies of hydrazine derivatives in this test system, mammalian metabolic activation was not used in this work. In addition, other details of the experimental procedure were difficult to ascertain in the publication. Purity and composition of the test substances were not provided, so that exact dosages could not be determined. Finally, no control values were provided as baselines for the positive results reported for the MH formulations; rather, the positive data were compared to the "spontaneous rate" of mutation. Apparently no concurrent controls were run.

d. Assays for DNA Damage/Repair, and Other Relevant Studies

Assays for DNA damage and/or repair in selected bacterial strains and studies in higher plants continue to register maleic hydrazide mutagenic, while comparable assays in animal test systems appear to perpetuate the considerable differential in sensitivity between the two kingdoms (Table II).

Thielmann and Gersbach's positive study (1978) of DNA-repair in two ether-permeabilized *Escherichia coli* strains and their repair deficient mutants (uvr, A and B; pol A-107), however, does not give sufficient detail on the composition of the maleic hydrazide used, nor on experimental and control procedures to be considered an adequate study. Other deficiencies include the lack of any mammalian (or other) metabolic activation, and the single concentration used (except in the case of the polymerase mutant, which remained refractory up to dosages as high as 450 μ M). The curious discrepancy between the inertness of this mutant (compared to the positive response of its isogenic (KMBL) wild-type strain), and the responsive uvr mutants (of strain H-540) is not adequately explained by the authors.

By contrast, in an equally sensitive assay, Griffen and Hill (1978) reported negative results for MH-induction (in vitro) of DNA-strand breakage in the colicinogenic plasmid, "E-1", carried by the *E. coli* K-12 strain, CR 34. However, these authors apparently used only one concentration (1 mg/ml) of an unidentified preparation of maleic hydrazide stated by them to have been provided by EPA's Pesticides and Toxic Substances Effects Laboratory at Research Triangle Park, North Carolina.

In the continuing series of studies from Kihlman's group at Uppsala relating to the mechanism of cellular repair processes, the report by Schubert and associates (1979) is interesting in several respects. That a 1×10^{-5} M concentration of maleic hydrazide produced both sister chromatid exchanges and chromatid aberrations in reconstructed karyotypic variants of *Vicia faba* was not unexpected and supports the compound's efficacy. What was most significant with the maleic hydrazide assays as well as with other mutagens tested was the apparent lack of correlation between intrachromosomal distribution patterns of the two cytogenetic effects. This further bolsters (for plants, at least) the notion among cytogeneticists that the occurrence and/or production of sister chromatid exchanges and chromosome aberrations are probably unrelated. However, were it not for the ample background on maleic hydrazide induced mutagenicity in higher plants, this study by itself would be less adequate than the questionable validity accorded it. Protocol deficiencies include insufficient data (for MH), with only one MH concentration employed, and the lack of identification of the test chemical.

Two mammalian DNA repair assays evaluated reaffirm the apparent resistance of animal cells to maleic hydrazide induced mutagenesis. The negative results that Stetka and Wolff (1976) reported in Chinese hamster cells treated with maleic hydrazide at 1×10^{-4} M and 1×10^{-3} M concentrations with/without a metabolic activation system (rat S-9) provide confirmation of the previous investigation of Perry and Evans (1975) in the same test system. Perry and Evans showed that maleic hydrazide treatments did not increase the frequency of sister chromatid exchanges in the chromosomes of Chinese hamster fibroblasts.

Finally, Seiler's (1977) attempt at validating Friedman and Staub's (1976) proposed short-term *in vivo* test (based upon inhibition of testicular DNA synthesis in mice) also tends to support other negative mammalian data for maleic hydrazide suggesting a rather low risk of maleic hydrazide induced mutagenic hazard to human or animal populations. The proposed test, however, is of questionable relevance *per se* because of lack of specificity to germ cells. In addition, Seiler's paper is not adequate to be entirely valid since there are too many deficiencies in reporting experimental details and results.

4. CONCLUSION

The Agency employs guidelines in assessing the mutagenic risk of any compound, the purpose of which is to ensure both quality and consistency of both testing and reporting. One of the primary focal points of the most recently proposed guidelines on risk assessment (45 FR 74984-74988, Nov. 13, 1980) is the weight-of-evidence approach. These proposed guidelines state that a judgement as to whether a chemical substance is likely to be a potential human mutagen will be based on a weight-of-evidence approach that considers the quality and adequacy of all available data.

Using these proposed guidelines for mutagenicity risk assessment to reassess the potential of maleic hydrazide as a human mutagen indicates that there is no reason to change the Agency's original position. Combining studies cited in Position Document 1 with those published after 1976 confirms that multitest evidence exists which would indicate maleic hydrazide may be a potential human mutagen. Multitest evidence, as defined in the guidelines, is a positive response in any two different point mutation test systems plus evidence indicating the presence of the test substance and/or its metabolite(s) in mammalian gonadal organs. However, while tests in several different test systems show that maleic hydrazide exhibits positive mutagenic effects, it is important to carefully assess the quality and adequacy with regard to multitest evidence.

Maleic hydrazide (MH Tech, K-MH and DEA-MH) has been tested in four separate systems: bacterial, plant, insect and mammalian (in vitro and in vivo). Valid and conclusive positive results indicate that maleic hydrazide is genetically active in certain strains of bacteria (B. megatherium, Northrop, 1963; B. Subtilis, Shiau, 1980).

Several valid studies also indicate potent genetic activity in plant cells; however, it is questionable whether this activity in plants is useful in judging potential mutagenicity to humans.

Valid, positive (Manna and Parida, 1972) and negative (Nawar and Hamza, 1978) studies have tested the mutagenic effects of maleic hydrazide in different insect systems. Other studies in insects are either invalid or inconclusive. In the absence of consistent, conclusive evidence, the mutagenic effects of maleic hydrazide in insects is considered suggestive but minimal.

Possibly, the ideal assessment of the mutagenic activity of a compound in humans can be ascertained by use of mammalian test systems. Several studies testing the effects of maleic hydrazide in mammalian systems have been performed. Of these, only two studies (Manna and Das, 1976; Nishi et al., 1979) reported positive results. The use of these two studies is questionable however, since the results of Manna and Das (1976) are considered invalid (Mauer, 1981) and those of Nishi (1979) are inconclusive (Mauer, 1981). Three valid mammalian studies (Stetka and Wolff, 1976; Bionetics, 1979; Paik and Lee, 1977) reported negative results. Other reported negative results are considered inconclusive (see Table II). From the weight-of-evidence concept set forth in the proposed mutagenicity risk assessment guidelines, it is reasonable to conclude that the genetic activity of maleic hydrazide in mammalian systems has not been demonstrated. Further, there are no studies

available that indicate either maleic hydrazide or its metabolites transports to mammalian gonadal tissue, thus providing indirect evidence of potential hereditary risk to humans.

In summary, multitest evidence still, according to proposed guidelines, exists for the mutagenic effects of maleic hydrazide. It is important to note, however, the quality and adequacy of the available data which constitutes multitest evidence. Overall, the data base, by virtue of the weight of evidence, appears to indicate that there is weak suggestive evidence supporting the positive mutagenic effects of maleic hydrazide and, at best, this data base is inconclusive. More important, however, only extremely weak suggestive evidence supports the contention that maleic hydrazide may be a mutagen in mammalian systems. Furthermore, evidence supporting a conclusion of serious risk for humans is weak because the data base is inconclusive and warrants development and review of additional studies.

Additional studies which were initiated (March, 1981) by Uniroyal Incorporated in response to the Agency's August 14, 1980 data request (FIFRA, Section (3)(c)(2)(B)) include:

- 1) Micronucleus Assay, using CD-1 random-bred mice.
- 2) Bacterial DNA Repair Test in Escherichia coli .
- 3) Mammalian Cell Mutation Assay, using L5178Y Mouse Lymphoma Cells.

All studies will examine the effects of the potassium formulation (K-MH) and will be reviewed when all requested toxicity tests have been completed.

*There are indications in one study (Oser, 1955) that DEA-MH but not Na-MH may have reached gonadal tissue to reduce fertility indices; however, it should be noted that DEA-MH produced after November, 1981 cannot be sold for use in the United States.

Table II. Mutagenicity Studies with Maleic Hydrazide (MH) Since Position Document I.

Genetic Effect/System	Test Assay	Reported Result	Reference	Comment
A) Gene Mutations:				
1) Microbial:				
(I)	Revertent plate assay in <i>S. typh.</i> TA 1530 exposed to 5, 10, 20 mg "pure" MH per plate or to 50, 100, 200 μ l ("neat") DEA-MH, K-MH	Negative: for MH Positive: for DEA-MH, K-MH inducing 5-8x "control" (spontaneous) frequency of revertants.	Tosk et al. 1979	Invalid
(II)	Revertent spot test in <i>B. subtilis</i> strain TKJ 6321 at 50 - 100 μ g MH tech, w/without metabolic activation	Positive: Increased freq. of revertants at least 2x spontaneous at 100 μ g without metabolic activation Negative: With rat S-9	Shiau et al., 1980	Valid
(III)	Forward mutation (to 8-Az+) and con. segregation (CO, ND) in <i>Aspergillus nidulans</i> exposed to homogenates from <i>N. alata</i> cult. cells treated with 2 mg (12 ml cultures).	Negative: MH not metabolized; no mutation induction.	Benigni et al., 1979	Invalid
(With plant metabolic activation)				
2) Insect:				
(I)	Sex-linked recessive lethals (SLRL) in progeny of adult <i>Drosophila mel.</i> males fed 4.46 or 44.6 mM tech MH.	Negative: No significant sex-linked recessive lethals increase in SLRL over control.	Navar & Hanz, 1978	Inconclusive

Table 11. Mutagenicity Studies with Maleic Hydrazide (MH) Since Position Document 1.

Genetic Effect/System	Test Assay	Reported Result	Reference	Comment
3) Insects:				
(1)	Dominant lethals in progeny of adult male/female D. mel. fed 4.46 or 44.6 μH tech. MH	Negative: No increase in dominant lethals over controls during 17 days (all 3 broods analyzed)	Nayar and Imanza, 1978	Valid
(11)	Struct. Chromosome aberrations scored in larval sal. glands of D. mel. exposed to 0.5% in feed	Positive: 10 inversions found in 6 larvae among a total of 4496 cells scored in 75 treated larvae, comp. to 0 (zero) among 5240 cells in 90 controls	Parkash and Miglani, 1978	Inconclusive
4) Mammalian Cells in Culture:				
(1)	Cytogenetic damage in V79 cells exposed to tech. MH (500-2000 $\mu\text{g}/\text{ml}$); K-MH (10,000 - 40,000 $\mu\text{g}/\text{ml}$); and DEA-MH (5,000 - 20,000 $\mu\text{g}/\text{ml}$), without metabolic activation.	Positive: Comp. to control (-4%), incr. conc.'s induced incr. freq. of cells with chromosome aberrations to max. of 18% at the LD_{50} for MH, K-MH, 13% at 2 X LD_{50} for DEA-MH	Nishi et al., 1979	Inconclusive
5) Mammals:				
(1)	Micronuclei in IM-PCF's of male mice given 100, 600 $\text{mg}/\text{kg}/\text{d}$ i.p. DEA-MH for two days (24 hr).	Negative: High dose ($=\text{LD}_{50}$) produced 2.44 ± 0.029 MCN, comp. with 3.08 ± 0.23 in vegetable oil control	Palk and Lee, 1977	Valid
(11)	Micronuclei in IM-PCF's of male mice given 100, 200 $\text{mg}/\text{kg}/\text{d}$ tech. MH twice	Negative: percentage of micronuclei at either dose no different from control.	Chaubey et al., 1978	Inconclusive

Table II. Mutagenicity Studies with Maleic Hydrazide (MH) Since Position Document 1.

Genetic Effect/System	Test Assay	Reported Result	Reference	Comment
B) Chromosome Aberrations:				
1) Lower Plants:				
(i)	Cytological effects in the eukaryotic alga, <i>Scenedesmus annulatus</i> at doses of 0.05% to 1.0% tech. MH	Negative: Only delay of mitotic division noted; MH toxic at doses above 0.5%	Sarma and Chaudhary, 1977	Invalid
2) Higher Plants				
(ii)	Cytogenetic damage in tip mitoses of <i>Viola faba</i> exposed to 1.5 X 10 ⁻⁴ M tech. MH for 1 hour	Positive: Post-R ₁ with 2.5 X 10 ⁻³ M Caffeine or Cl-Caf. further incr. chromosome aberrations of MH by 7-8x	Sturelid and Kihlman, 1975	Invalid
(iii)	Micronuclei in tetrads of <i>Tradescantia paludosa</i> FHC's treated with 5 conc.'s of tech. MH (8.9 - 17.9mM)	Positive: Increased micronuclei per tetrad, 1.7 to 2.0X over control	Ma, 1981	Invalid
(iiii)	Cytogenetic damage in root-tip mitoses of <i>V. faba</i> and <i>Allium cepa</i> w/without Caf. at 1.5 X 10 ⁻³ M tech MH in 0.007M Sorensen-Po ₄ buffer	Positive: Compared to 0 (zero) in control, 17% abnormal cells with 18.5% chromosome aberrations	Hartley-Asp 1976	Inconclusive
(iv)	Cytogenetic damage in root-tip mitoses of <i>A. cepa</i> to 10 ⁻³ , 10 ⁻⁴ M 10 ⁻⁵ M MH, at 15°C and 22°C	Positive Compared to controls (2%), incr. freq. (to max. of 30%) cells with CA (most chromatid), directly prop. to MH conc. and temp. for both metaphase and anaphase preps.	Oku, 1977	Valid

Table 11. Mutagenicity Studies with Maleic hydrazide (MH) Since Position Document 1.

Genetic Effect/System	Test Assay	Reported Result	Reference	Comment
C) DNA Damage/Repair				
1) Bacterial				
(1)	Excision-repair synth. in two strains of ether-treated E. coli cells and their repair-def. mutants exposed to 300 μ M (115/60 and uvr mutants) or 30 μ M (KML and pol-A) tech. MI in tris-HCl buffer	Positive: Comparable repair in wild-type H-540 as in uvr mutants. Contrast to repair in only wild-type KML (1978) but not in polA (1985).	Thielmann and Garabach, 1978	Invalid
2) Plants				
(1)	Sister chromatid exchanges in root-tip mitoses of Vicia faba chromosomal variants (comp. to CA), treated with 10-5 M tech. MI.	(7) Positive: Treated had 3 X the spont. SCE per/cell (75 vs 25); but no correlation with distrib. of chromosome aberrations produced in 15% of cells	Schubert et al, 1979	Inconclusive
3) Mammalian Cells in Culture				
(1)	Sister chromatid exchanges in CHO cells treated with 10-4, 10-3 M tech. MI.	Negative: No increase in sister chromatid exchanges in treated cells.	Stetka and Wolff, 1976	Valid

Table II. Mutagenicity Studies with Maleic Hydrazide (MH) Since Position Document 1.

Genetic Effect/System	Test Assay	Reported Result	Reference	Comment
D) Other Relevant Studies:				
1) DNA Strand Breakage (1)	Breakage of DNA strands in colicinogenic plasmid, E-1, carried in E. coli K12 strain CR 34 treated at 1.0 mg/ml tech. MH in DMSO.	Negative: No induction of strand breakage in treated E-1	Griffen and Mill, 1978	Inconclusive
2) DNA Synthesis in Mice: (1)	Inhibition of DNA synthesis in testicular tissue of mice given 1000 mg/kg tech. MH p.o.	Negative: No inhibition at one dose tested.	Seller, 1977	Inconclusive

III. DECISION

A. DECISIONS AND SUPPORTING RATIONALE

This decision document is a comprehensive reassessment of potential risks associated with maleic hydrazide. The purpose of this document is to make a decision based not only on the reevaluation of supporting information cited in PD 1, but also on analyses of newer information that was received after publication of PD 1. After thoroughly reviewing each area of concern it has been determined that two of the three initial RPAR triggers (oncogenicity and reproductive effects) are not supported. This determination is based on reassessment of original supporting data in PD 1 and further supported by additional studies which became available for consideration since publication of PD 1. In addition, the individual conclusions for each area of concern (oncogenicity, mutagenicity and reproductive effects) are based on the criteria set forth in FIFRA Section (3)(c)(8), which require validated tests or other significant evidence to demonstrate that a compound will cause unreasonable adverse risk. The PD 1's initial concern for oncogenicity and reproductive effects were found not to be supported by valid tests, or significant evidence. The third trigger, mutagenicity, still stands based on multitest criteria set forth in the proposed mutagenicity guidelines. Only extremely weak suggestive evidence, however, supports the contention that maleic hydrazide is mutagenic in mammalian systems. Furthermore, evidence supporting a conclusion of serious risk for humans is weak because the data base is inconclusive and thus warrants development and review of additional studies.

Based on the reassessment of the initial RPAR triggers and the results of requests for additional data, the Agency has reached several decisions.

First, the current RPAR investigation of maleic hydrazide will be inactivated until or unless new evidence arises which raises prudent concerns of potential unreasonable adverse risks to man or the environment. The Agency will review the new data requested from registrants when they are submitted to determine whether prudent concerns may arise.

Second, based on available toxicity data, the Agency will allow continued use of potassium maleic hydrazide registrations, provided the registrants continue to comply with previous data requests.

Third, the Agency is establishing a limit to the amount of hydrazine contaminant permitted in MH products in order to avoid the potential for unreasonable adverse effects. The acceptable limit is 15 ppm hydrazine in technical grade products, because 15ppm is the highest level reported in currently registered products and because the potential risk associated with that level is very low. Registrants of current technical products have promised to meet this standard and must now update their confidential statements of formula.

Incidental to these decisions, it should be noted that all diethanolamine maleic hydrazide (DEA-MH) registrations were suspended in November, 1981, based on an industry-wide decision not to initiate studies requested by EPA on this formulation. This action stopped production of DEA-MH for sale in the United States, but allowed continued sale of existing stocks. The suspension may be lifted at any time if any registrant wishes to generate the requested additional data.

B. ADDITIONAL DATA REQUIREMENTS

The Agency made a previous decision that it lacked adequate data to determine whether products containing either the diethanolamine or potassium salt causes unreasonable adverse effects on humans. This determination was the basis of a request for additional mutagenic and reproductive effects data which was sent to all maleic hydrazide registrants on August 14, 1980. The rationale for requesting additional data was:

1. The Agency is aware of only one reproductive effects study (Oser, 1955). From the data presented in this study, the Agency concluded that a daily diet containing 0.1% DEA-MH causes pronounced adverse reproductive effects in rats. Such a diet is equivalent to a daily ingestion of 100 mg/kg of DEA-MH. The Agency's primary concern is that because only one non-toxic dose level was used in this study, neither a no-observed-effect-level nor a dose-response relationship could be determined. Thus, the Agency was unable to determine whether an ample margin of safety exists as to the reproductive effects produced by DEA-MH.

2. While the Oser study is not valid for the purpose of supporting an RPAR trigger, it is nonetheless suggestive of possible adverse reproductive effects for DEA-MH. Therefore, additional data were requested to establish a NOEL for this formulation. Furthermore, the Agency has no data regarding the reproductive effects of K-MH.

3. In order to resolve various mutagenic effects issues, the Agency has determined that further studies are needed. The issue of induction of sex-linked recessive lethal mutations in Drosophila melanogaster is unresolved since both positive and negative results have been reported. Chromosome breaks in plants, insects and mice have been reported; however, issues such as differential sensitivities between plants and animals as well as the potential for genetic hazard to humans need be resolved. The following list (drawn from the proposed FIFRA testing guidelines as found in 43 FR 163:37388, August 23, 1978) was presented to registrants as acceptable types of mutagenicity testing that would satisfy the mutagenicity data requirements of the August 14, 1980 Notice and Order:

- a. bacterial point mutation studies
- b. eukaryotic microorganism point mutation studies
- c. mammalian somatic cell in culture point mutation studies
- d. DNA repair in bacteria

- e. unscheduled DNA repair synthesis in mammalian cells
- f. mitotic recombination and/or gene conversion in yeast
- g. sister chromatid exchange in mammalian cells
- h. sex-linked recessive lethal test in insects
- i. in vivo cytogenetics in mammals
- j. insect tests for heritable chromosomal effects
- k. dominant lethal effects on rodents
- l. heritable translocation tests in rodents
- m. mouse specific locus test

The legal basis for additional data requests is FIFRA Section (3)(c)(2)(B). Failure to comply with this request allows the Administrator to suspend pesticide registrations.

BIBLIOGRAPHY

- Andersen, K.S., E. G. Leighty, and M.T. Takahashi. 1972. Evaluation of herbicides for possible mutagenic properties. *J. Agr. Food Chem.* 20(3): 649-655.
- Anderson, L.D., 1981. Attachment to the Carcinogen Assessment Group's (CAG) updated evaluation of maleic hydrazide concerning hydrazine unit risk.
- Barnes, J.M., P.N. Magee, E. Boyland, A. Haddow, R.D. Parsey, W.S. Bullough, C.N.D. Cruickshank, M.H. Salaman, and R.T. Williams. 1957. The nontoxicity of maleic hydrazide for mammalian tissues. *Nature* 180:62-64.
- Benigni, R., M., Bignami, I. Camori, A., Carere, G., Conti, R., Iachetta, G., Morpugo and V.A. Ortali (1979) A new in vitro method for testing plant metabolism in mutagenicity studies. *J. TOXICOL. ENVIRON. HLTH.* 5:809-819.
- Biancifiori, C. 1970. Hepatomas in CBA/Cb/Se mice and liver lesions in Golden hamsters induced by hydrazine sulfate. *J. Natl. Cancer Inst.* 44:943-953.
- Bionetics. 1978. Sex-linked Recessive Lethal Assay in *Drosophila*. Evaluation of maleic hydrazide (potassium salt) chemical in distilled water (26.78% w/v). Final report submitted to Uniroyal Chemical by Litton Bionetics Inc. (LBI Project No. 20906, February 22, 1978).
- Cabral, R., V. Ponomarev, G. Van Esch. 1980. Report in 1980 WHO Annual Report (untitled, study complete but not yet published) and personal communication with G. Thies, 1981.
- Carcinogen Assessment Group (CAG). 1977. Assessment of the carcinogenicity of maleic hydrazide.
- Carcinogen Assessment Group (CAG). 1981. Updated evaluation of the carcinogenicity of maleic hydrazide.
- Chaubey, R.C., B.R. Kavi, P.S. Chauhan and K. Sundarsam (1978) The effect of hycanthone and maleic hydrazide on the frequency of micronuclei in the bone-marrow erythrocytes of mice. *MUTATION RES* 57:187-191.
- Chen, C. 1981. Memorandum for the record concerning statistical analysis of two studies dealing with teratogenic and adverse reproductive effects of maleic hydrazide. 1 page.
- Dickens, F., and H.E.H. Jones. 1965. Further studies on the carcinogenic action of certain lactones and related substances in the rat and mouse. *Brit. J. Cancer* 19:392-403.
- Epstein, S.S., E. Arnold, J. Andrea, W. Bass, and Y. Bishop. 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol. Appl. Pharmacol.* 23:288-325.
- Epstein, S.S., and N. Mantel. 1968. Hepatocarcinogenicity of the herbicide maleic hydrazide following parenteral administration to infant Swiss mice. *Int. J. Cancer* 3:325-335.

- Felkner, I.C. 1979. Rebuttal analysis of maleic hydrazide (MH) --- Area of concern: Mutagenicity. Submitted to EPA, May 20, 1979.
- Farm Chemical Handbook. 1979. Meister publishing. Willoughby, Ohio.
- Friedman, M.A. and J. Staub. 1976. Inhibition of mouse testicular DNA synthesis by mutagens and carcinogens as a potential simple mammalian assay for mutagenesis. *MUTATION RES.* 37:67-76.
- Griffin, D.E. III and W.E. Hill. 1978. *In vitro* breakage of plasmid DNA by mutagens and pesticides. *MUTATION RES.* 52:161-169.
- Hartley-Asp, B. 1976. Enhancement by methylated oxypurines of the frequency of induced chromosomal aberrations. IV. The dependence of the potentiation on the type of material. *HEREDITAS* 83:223-236.
- Hill, R. 1977. Memorandum concerning maleic hydrazide: mammalian reproduction study. 1 page.
- Hitachi, M.K. Yamada, and S. Takayama. 1975. Brief communication: Cytologic changes induced in rat liver cells by short-term exposure to chemical substances. *J. Nat. Cancer Inst.* 54(5):1245-1247.
- Hunter, B., L.E. Mawdesley-Thomas, and A.N. Worden. 1973. The administration of maleic hydrazide and its diethanolamine salt to rats. *Toxicology.* 1:301-307.
- Innes, J.R.M., B.M. Ulland, M.G. Valeria, L. Petrucelli, L. Fishbein, E.R. Hart, and A.J. Pallotta (Bionetics Research Laboratories, Inc.) and R.R. Bates, H.L. Falk, J.J. Gart, M. Klein, I. Mitchell, J. Peters (National Cancer Institute). 1969. Bio-assay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. *J. Natl. Cancer Inst.* 42:1101-1114.
- International Research and Development Corporation. 1979. Twelve-month interim report of a lifetime oncogenicity study on maleic hydrazide (potassium salt) in mice.
- Kak, S.N., and B.L. Kaul. 1975. Mutagenic activity of hydrazine and its combinations with maleic hydrazide and x-rays in barley. *Cytobios.* 12: 123-128.
- Khera, K.I., C. Walen, G. Trivett, and G. Angers. 1979. Teratogenic assessment of maleic hydrazide and daminozide, and formulations of ethoxyquin, thiabendazole and naled in rats. *J. Environ. Sci. Health, Part B* 14:563-577.
- Ma, T.H. and V.A. Anderson. 1979. Micronuclei induced by ultraviolet light and chemical mutagens. *ENVIRON. MUTAG.* 1:123-124.

- Ma, T.H. 1980. Chromosome breakage induced by maleic hydrazide in cultured human lymphocytes and Tradescantia pollen mother cells. ENVIRON. MUTAG. 2:287 (Abstract).
- Ma, T.H. 1981. Tradescantia MCN-in tetrad Mutagen Test -- On site monitoring and further validation. (Final Report on Grant #R-806422010, HERL, US-EPA, RTP: Project Officer, S.S. Sandhu, MD-68, HERL, January 1, 1981.
- Manna, G.K., R.K. Das, and P.K. Das. 1976. Chromosome aberrations in mice treated with maleic hydrazide, uracil and guanine. Nucleus 19(i):40-46.
- Manna, G.K. and B.B. Parida. 1972. Differential maleic hydrazide (MH) sensitivity shown by the spermatocyte chromosomes in five species of grasshopper. Cytologia 37(2):345-353.
- Mannell, W.A., and H.C. Grice. 1975. The effects of maleic hydrazide and p-dimethylaminoazobenzene on rat liver. Can. J. Biochem. Physiol. 35: 1233-40.
- Mauer, I. 1981. Memorandum: Mutagenicity update of maleic hydrazide (with two emendations).
- McCann, J., E. Choi, E. Yamasaki, and B.N. Ames. 1975. Detection of carcinogens as mutagens in the Salmonella microsome test: Assay of 300 chemicals. Proc. Nat. Acad. Sci. 72(12):5135-5139.
- McCarthy, R.E. and S.S. Epstein. 1968. Cytochemical and cytogenetic effects of maleic hydrazide on cultured mammalian cells. Life Sciences. 7:1-6.
- Mukhorina, K.V. 1962. Effect of maleic hydrazide, which inhibits the germination of the plant, on animal organisms. Gig. Tox. 20:156-164.
- Nasrat, G.E. 1967. The effect of pre-treatment with maleic hydrazide of the mutation rate induced by gamma radiation in Drosophila melanogaster. JAP. J. GENETICS 42:39-42.
- Nawar, M.M. and H.A. Hamza. 1978. Influence of maleic hydrazide mixed with ethylemethanesulfonate on mutation frequencies induced in Drosophila melanogaster. EGYPT. J. GENET. CYTOL. 7:69-78.
- Nishi, Y.M., Mori and N. Inui. 1979. Chromosomal aberrations induced by maleic hydrazide and related compounds in Chinese hamster cells in vitro. MUTATION RES. 67:249-257.
- Northrup, J.H. 1963. Studies of the origin of bacterial viruses: VII. The effect of various mutagens (urethane, ethyl urethane, hydrogen peroxide, oesoxycholate, maleic hydrazide, butadiene dioxide, triethylene melamine, versene, and acriflavin) on the proportion of virus-producing and streptomycin-resistant cells in cultures of B. megatherium 20. J. Gen. Physiol. 46:971-980.
- Oser, B. 1955. Toxicological studies of chemical additives. 3 Maleic hydrazide (1,2-dihydropyridazine)3,6 dione. Food Research Laboratories, Long Island, New York.

- Paik, S.G. and S.Y. Lee. 1977. Genetic effects of pesticides in mammalian cells. I. Induction of micronucleus. KOREAN J. ZOOL. 20:10-29.
- Parkash, R.A and G.S. Miglani. 1978. Hydroxylammonium sulphate and maleic hydrazide induced chromosomal aberrations in Drosophila. CARYOLOGIA 31: 1-5.
- Perry, P. and H.J. Evans. 1975. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange . Nature 258:121-125.
- Plewa, M.J. 1978. Activation of chemicals into mutagens by green plants: A preliminary discussion. ENVIRON. HLTH. PERSP. 27:45-50.
- Raltech. Raltech Scientific Services. 1981. Protocol TP-109, Two generation rat reproduction study. Ralston Purina Company Madison, Wisconsin
- Scott, B.R., A.H. Sparrow, S.S. Schwemmer and L.A. Schairer. 1978. Plant metabolic activation of 1,2-dibromoethane (EDB) to a mutagen of greater potency. MUTATION RES. 49:203-212.
- Schubert, I., S. Sturelid, P. Dobel and R. Rieger. 1979. Intrachromosomal distribution patterns of mutagen-induced SCE's and chromatid aberrations in reconstructed karyotypes of Vicia faba. MUTATION RES. 59:27-38.
- Seiler, J.P. 1977. Inhibition of testicular DNA synthesis by chemical mutagens and carcinogens. Preliminary results in the validation of a novel short term test. MUTATION. RES. 71:169-179.
- Shiau, S.Y., R.A. Huff, B.C. Wells and I.C. Felkner. 1980. Mutagenicity and DNA-damaging activity for several pesticides tested with Bacillus subtilis mutants. MUTATION RES. 71:169-179
- Stetka, D.G. and S. Wolff. 1976. Sister chromatid exchange as an assay for genetic damage induced by mutagen carcinogens. II. In vitro test for compounds requiring metabolic activation. MUTATION RES. 41:343-350.
- Sturelid, S. and B.A. Kihlman. 1975. Enhancement by methylated oxypurines of the frequency of induced chromosomal aberrations. 2. Influence of temperature on potentiating activity. HEREDITAS 80:233-246.
- Szybalski, W. 1958. Special microbiological systems. II. Observations on chemical mutagenesis in microorganisms. Annu. N.Y. Sci. 76:475-489.
- Thielmann, H.W. and H. Gersbach. 1978. The nucleotide-permeable Escherichia coli cell, a sensitive DNA repair indicator for carcinogens, mutagens, and antitumor agents binding to DNA Z. KREBSFORSCH. 92:177-214.
- Thies, G.A. 1981(a) Personal communication with Marshall Thomas, General Manager, Fairmount Chemical Company, Lexington, Kentucky.
- Thies, G.A. 1981(b) Personal communication with Dr. R. Cabral, International Agency for Research on Cancer, Lyon, France

Tosk, J., I. Schmeltz and D. Hoffman. 1979. Hydrazines as mutagens in a histidine-requiring auxotroph of Salmonella typhimurium. MUTATION RES. 66:247-252.

Van Der Heijden, C.A. Engelina M. Den Tonkelaar, Johanna M. Garbis-Berkvens and G.J. Van Esch. 1981. Maleic hydrazide, carcinogenicity study in rats. Toxicology, 19:139-150.